

EXHIBIT 1



1.800.340.1633

Main Site

CD
Products &
Services

DVD
Products &
Services

Ordering
Process

About
OneOff

History of CD Technology



History of OneOff

Mission Statement

History of CD
Technology

- 1841 Augustin-Louis Cauchy Proposes a Sampling Theorem.
- 1842 Charles Babbage Proposes analytical engine for performing and storing calculations.
- 1854 George Boole publishes "An Investigation Into the Laws of Thought." A book that contained, among other things, theories that were later used to build digital circuits.
- 1855 Leon Scott de Martinville invents the phonoautograph, a machine that records vibrations on a carbonized paper cylinder.
- 1876 Alexander Graham Bell introduces the telephone
- 1877 Thomas Edison invents the phonograph while trying to invent a device that would record and repeat telegraphic signals (digital)
- 1887 Emily Berliner replaces Edison's wax cylinder phonograph with the audio disc.
- 1915 78 R.P.M records introduced
- 1922 J.R. Carson examines the idea of time sampling for communications
- 1928 Harry Nyquist publishes "Certain Topics in Telegraph Transmission Theory." His theory contained proof that the technology used in today's audio cd's could work.
- 33 1/3 Records Introduced
- 1937 A. Reeves invents pulse code modulation (PCM), a technology used by computers and CD's for audio in the present day.
H. Aiken from Harvard approaches IBM and proposes a electrical computing machine.
- 1943 The U.S. Army turns on the first computer (ENIAC) at the University of Pennsylvania.
- 1947 Magnetic Tape Recorders hit the U.S. market.
- 1948 The transistor is invented by Bell Laboratories.
Claude E. Shannon publishes "A Mathematical Theory of Communication." -- Yet another important development for theories used in CD technology
- 1949 45 rpm records hit the U.S. market, thanks to microgroove technology.
- 1950 Richard W. Hamming publishes information about error detection/correction codes. It would be impossible for CD's to work without error correction.
- 1958 Invention of the Laser.
Stereo LP's produced.
Integrated Circuit introduced by Texas Instruments
- 1960 Computer Music experiments take place at major laboratories.
I.S. Reed and G. Solomon publish information on multiple error correction codes. These come to be known as the "Reed-Solomon"

Codes which are the codes used for encoding and reading CD's.
Working Laser produced.

- 1967 NHK Technical Research Institute demonstrates a 12-bit PCM digital audio recorder with a 30 kHz (30,000 times per second) sampling rate. The digital recording goes onto a high-grade video tape.
- 1969 Sony introduces it's 13-bit PCM digital recorder at a 47.25 kHz (47,250 time per second) sampling rate. The digital recording is sent to a 2" video tape.
Klass Compaan, a Dutch physicist comes up with the idea for the Compact Disc.
- 1970 At Philips, Compaan and Pete Kramer complete a glass disc prototype and determine that a laser will be needed to read the information.
- 1971 Microprocessor produced by Intel
Digital Delay line used by BBC's studios (first digital audio device).
- 1972 Compaan and Kramer produce color prototype of this new compact disc technology
- 1973 BBC and other broadcast companies start installing digital recorders for master recordings.
- 1977 Mitsubishi, Hitachi & Sony show digital audio disc prototypes at the Tokyo Audio Fair.
JVC Develops Digital Audio Process
- 1978 Philips releases the video disc player
Sony sells the PCM-1600 and PCM-1 (digital audio processors)
"Digital Audio Disc Convention" Held in Tokyo, Japan with 35 different manufacturers.
Philips proposes that a worldwide standard be set.
Polygram (division of Philips) determined that polycarbonate would be the best material for the CD.
Decision made for data on a CD to start on the inside and spiral towards the outer edge.
Disc diameter originally set at 115mm.
Type of laser selected for CD Players.
- 1979 Prototype CD System demonstrated in Europe and Japan.
Sony agrees to join in collaboration.
Sony & Philips compromise on the standard sampling rate of a CD -- 44.1 kHz (44,100 samples per second)
Philips accepts Sony's proposal for 16-bit audio.
Reed-Solomon code adopted after Sony's suggestion.
Maximum playing time decided to be slightly more than 74 minutes.
Disc diameter changed to 120mm to allow for 74 minutes of 16-bit stereo sound with a sample rate of 44.1 kHz
- 1980 Compact Disc standard proposed by Philips & Sony.
- 1981 Matsushita accepts Compact Disc Standard
Digital Audio Disc Committee also accepts Compact Disc Standard.
Sharp achieves production of semiconductor laser.
Philips & Sony collaboration ends.
- 1982 Sony & Philips both have product ready to go.
Compact Disc Technology is introduced to Europe and Japan in the fall.
- 1983 Compact Disc Technology is introduced in the United States in the

- spring
The Compact Disc Group formed to help market.
CD-ROM Prototypes shown to public
30,000 Players sold in the U.S.
800,000 CD's sold in the U.S.
- 1984 Second Generation & Car CD players introduced.
First Mass Replication Plant in the United States built.
Portable (i.e., Sony DiscMan) CD Players sold.
- 1985 Third generation CD Players released.
CD-ROM drives hit the computer market.
- 1986 CD-I (Interactive CD) concept created.
3 Million Players sold in U.S.
53 Million CD's sold in U.S.
- 1987 Video CD format created.
Allen Adkins of Optical Media International joins with SonoPress in
Amsterdam and demonstrates a desktop system for pre-mastering CD's
(Adkins and SonoPress, produced a replicated CD in less than 24-hours
using this system).
- 1988 CD-Recordable Disc/Recorder Technology Introduced
- 1990 28% of all U.S. households have CD's.
9.2 million players sold annually in the United States.
288 million CD's sold annually in the United States.
World Sales close to 1 Billion
- 1991 CD-I format achieved.
CD-Recordable Introduced to the Market
"QuickTopix" the first CD-R pre-mastering Software introduced by
Allen Adkins.
- 1992 CD-R Sales reach 200,000
- 1996 DVD Technology Introduced.
Prices of Recorders and CD-R Media go down significantly.
High Demands cause World-Wide CD-R Media Shortage.
- 1997 DVD Released.
DVD Players/Movies hit consumer market.
DVD-R standard created (3.9 Gig).
Mitsui builds it's first CD-R production plant in the U.S.
World-wide shortage ends.
Price of CD-R media lower than ever imagined.
- 1998 DVD-RAM, DVD-Recordable systems/equipment hits market.
DVD-Video/ROM authoring tools hits the market.
CD-R prices continue to drop.
- 1999 DVD-Video Becomes main stream.
Consumers begin purchasing DVD Players & Movies on a mass level.
Most major film studios have titles on DVD.
DIVX Dies (DIgital Video eXpress).
Second Generation DVD Burners.
4.7 Gig DVD-R Media Developed.

Source

1841-1991

1991-1999

Pohlmann, Ken C.
"The Compact Disc Handbook, 2nd
Edition" (Click to See this Book at
Amazon.Com)
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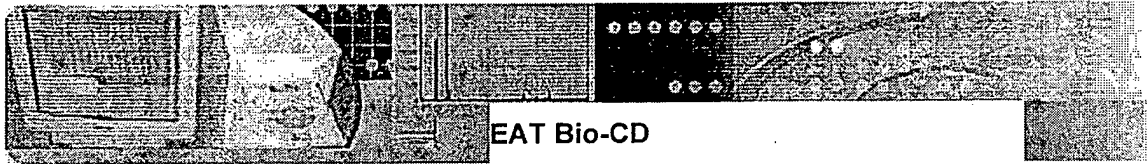
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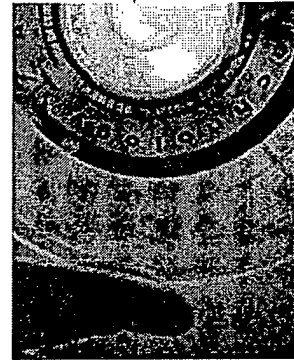
EXHIBIT 2



Combining biological and digital information on the same surface

Main features of the Bio-CD:

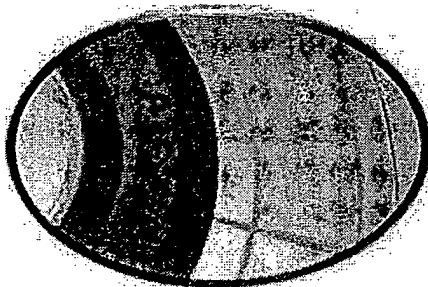
- Up to 20 arrays per disc reducing cost per array
- Convenient for both research and diagnostic applications
- Ultra-sensitive colorimetric detection using EAT Silverquant® technology
- Results of analysis stored on Bio-CD recordable track



Bio-CD: microarrays on compact disc

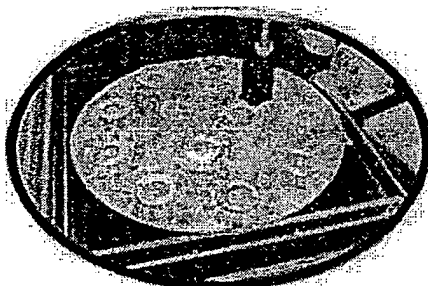
Full technology platform has been developed

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Close view of arrays on CD

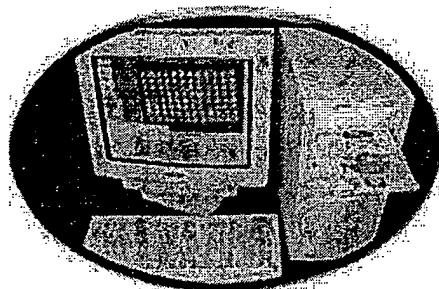
Functionalization of the Bio-CD using proprietary resins



Bio-CD arrayer

Bio-CD arrayer: custom CD-spotter having a capacity of 20 discs

Bio-CD Reader: quantify and burns biological datas on the recordable track of the Bio-CD



Bio-CD reader

Please click [here](#) for an overview of the Bio-CD reader

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Related publication: Compact Disc with Both Numeric and Genomic Information as DNA microarray platform, I. Alexandre, Y. Houbion, J. Collet, S. Hamels, J. Demarteau, J-L Gala, BioTechniques 33-435-439, 2002 / 08

This technology and the products are available for licensing in several applicational areas.

Your licensing and OEM contact:

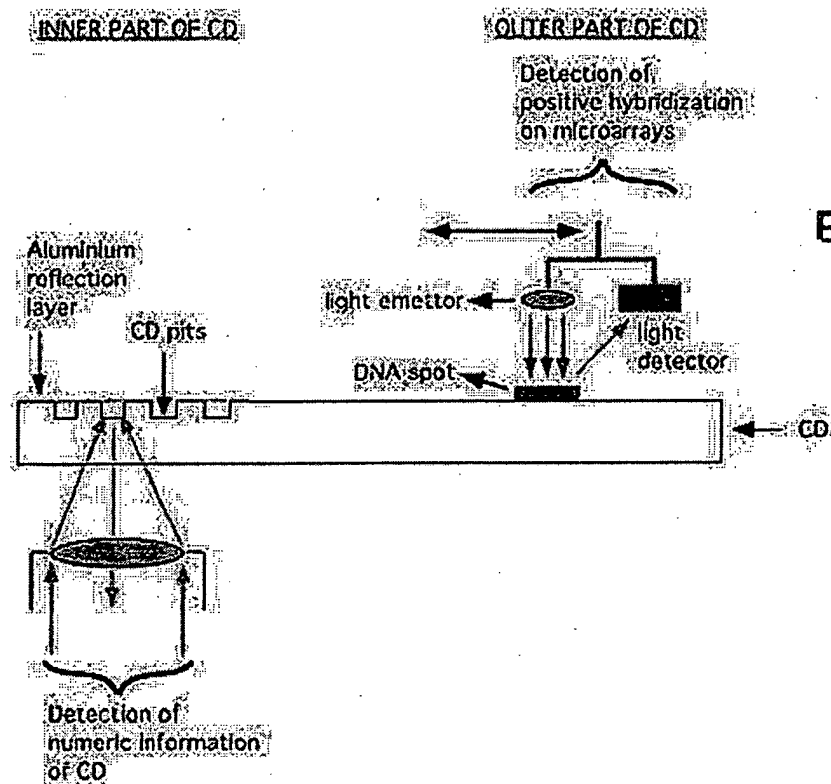
Jürgen Lindemeier, Ph.D.

Eppendorf AG

Barkhausenweg 1

22339 Hamburg

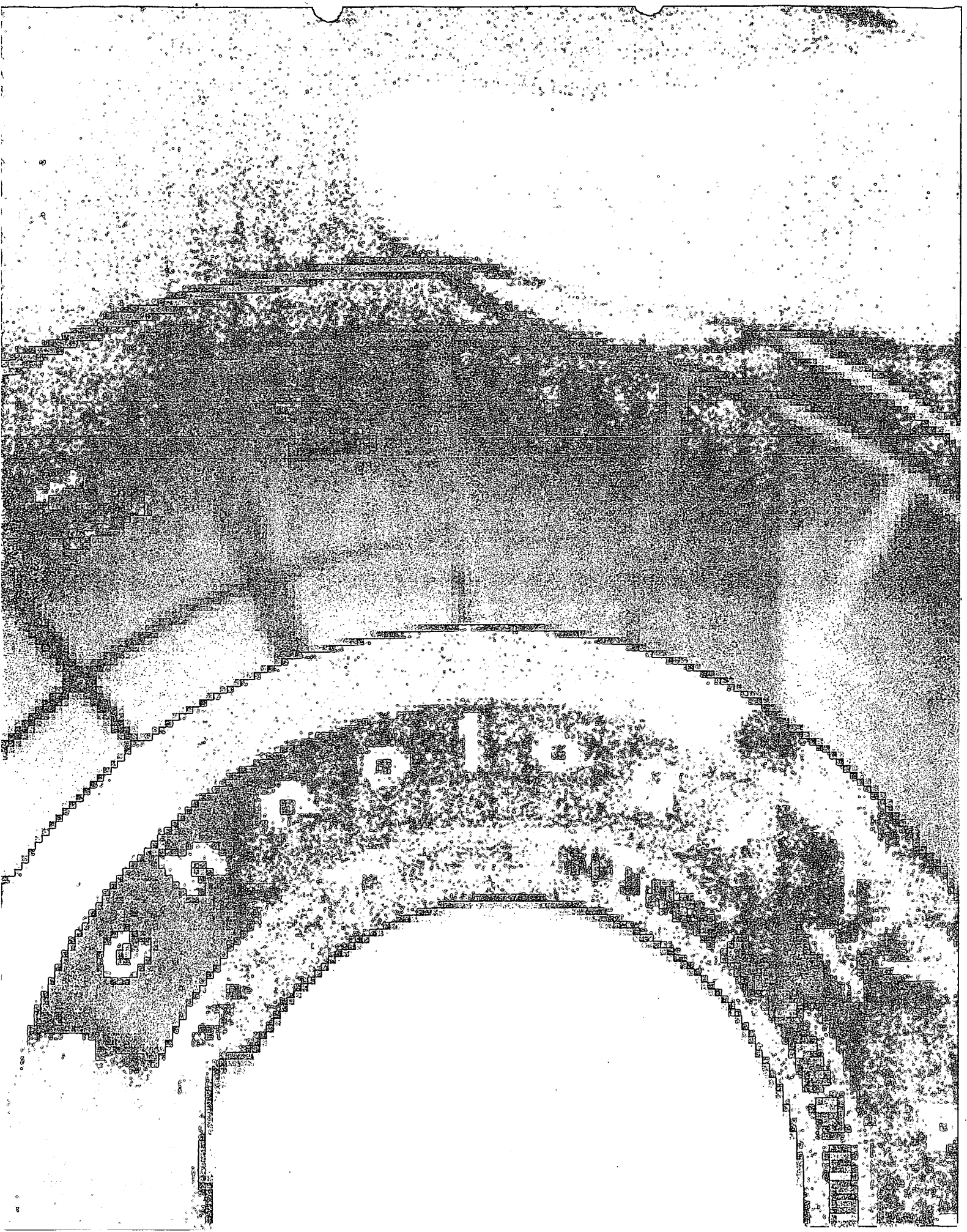
lindemeier.j@eppendorf.de



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Design of the Bio-CD reader

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PRODUCT APPLICATION FOCUS

A forum for manufacturers to describe the current and potential applications of new research instruments or products.

Compact Disc with Both Numeric and Genomic Information as DNA Microarray Platform

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FUNDP and ¹Advanced Array Technology (AAT), Namur, and ²Saint-Luc University Hospital, Brussels, Belgium

BioTechniques 33:435-439 (August 2002)

ABSTRACT

The compact disc (CD) is an ideal tool for reading, writing, and storing numeric information. It was used in this work as a support for constructing DNA microarrays suited for genomic analysis.

The CD was divided into two functional areas: the external ring of the CD was used for multiparametric DNA analysis on arrays, and the inner portion was used for storing numeric information. Because polycarbonate and CD resins autofluoresce, a colorimetric method for DNA microarray detection was used that is well adapted for the fast detection necessary when using a CD reader. A double-sided CD reader was developed for the simultaneous analysis of both array and numeric data. The numeric data are engraved as pits in the CD tracks and result in the succession of 0/1, which results from the modulation of the laser reflection when one reads the edges of the pits. Another diffraction-based laser was placed above the CD for the detection of the DNA targets on the microarrays. Both readers fit easily in a PC tower. Both numeric and genomic information data were simultaneously acquired, and each array was reconstituted, analyzed, and processed for quantification by the appropriate software.

INTRODUCTION

Miniaturization has been a main target of the electronic industry and is now invading molecular biology through the technology of microarrays or biochips (3-6). The principle of DNA microarray analysis was known for many years as reverse dot blot analysis, which was first performed on large nitrocellulose or nylon membranes. Efforts in miniaturization were associated with new supports. The electronic chips were first proposed for DNA binding using electric-based targeting (9). The possibility of in silico synthesis of nucleotide sequences combined with photolithography provided surfaces with several thousand different small oligonucleotide capture

probes (11,12). Glasses were also activated by introducing aldehyde groups for the covalent binding of aminated DNA capture probes. The miniaturization was obtained for this method through the use of robotic precise automatic spotting (13). In all cases, the hybridized DNA is detected by incorporating fluorescent labels either directly during the copy of target sequences to be analyzed or by a second labeling step with fluorescent streptavidin or antibodies (7,9). The search for electronic-based detection methods (10,11) continues, but the proposed solutions are now becoming of practical use (14).

The search for how to use polymers such as polycarbonate for supporting DNA microarrays was hampered mainly because of their autofluorescence. However, a new colorimetric method has been proposed, based on the deposit of silver precipitation at the DNA location (1,10). This new method allows plastic polymers to be tested as support for the DNA arrays. Although the three-log dynamic range of the colorimetric detection method is lower than the four-log range of the Cy3 fluorescence method, the silver precipitation colorimetric method is as sensitive as the Cy3 fluorescent detection of DNA when it is performed on glass slides (1).

Here we used compact disc (CD) as a support for constructing DNA assays. The goal of the project was to use a common platform for storing numeric and genomic data, both being read by one or two reading devices inserted into a PC tower. The first attempt (data not shown) was to take part of the CD technology developed for reading and engraving the numeric information, using the laser reflection process present in a normal CD reader. Indeed, we succeeded in transferring the DNA hybridization site onto the site of numeric information and read it with a normal CD reader, but the solution was impractical because of the constraint of working in a clean atmosphere. We then decided to separate physically on the CD the location of the encoded numeric information from the DNA binding location. This solution gave the best results and is described in this paper.

MATERIALS AND METHODS

Bio-CD

The commercially available CD is a 1.2-mm-thick polycarbonate disc that carries on its upper side one track running from the internal to the external part of the disc. The track is composed of pits that are 1–4 μm long, 0.15 μm deep, and 0.5 μm wide (2). This upper surface is then covered by a reflective aluminium or gold layer that is then protected from oxidation by a varnish layer.

The pits give binary information in a simple way: a laser beam is focused on the surface of the pits and, when the beam is reflected by a flat surface, gives a 0 value. However, when the reflection meets the edge of a pit, it decreases below a threshold value and this deflection is counted as a 1 value. The succession of the 1/0 numeric signals are then converted into data of different kinds like bytes for computers, some of them necessary for the CD reader to recognize the CD, adjust the speed of rotation, and control the position of the head compared to the CD. The reader follows the track as a continuous spiral on the CD with an elaborate servo tracker (part of the CD reader) that corrects for both lateral and vertical variations.

To avoid interference between the gene-based signal and the numeric reading that also provides the track and controls the speed, we separated the two signals laterally on the CD and detected them separately with two laser-reading devices. For the lateral separation, the CDs were engraved with a numeric information band of around 1 cm located on the inner part of the CD and covered with an aluminium layer restricted to this location. The outer part was a transparent polycarbonate band coated with a DNA fixation layer. This specially designed CD for microarray technology is called the Bio-CD (Figure 1).

Bio-CD for *Staphylococcus* Detection

The DNA spotting, PCR amplification, and hybridization were performed as described previously (8). In brief, the *femA* gene of the various *Staphylococci* are amplified by a consensus set of primers and then detected on CD microarrays that bear the capture probes specific for the *femA* of the dif-

ferent *Staphylococci* species. Array spotting on the CD was performed on an arrayer with a plate that could support 12 CDs (Figure 2).

The spots were 300 μm in diameter, and the DNA bound to the CD through the use of a specific fixation layer coated on the CD (UCB, Drogenbos, Belgium).

Colorimetric Silver Labeling

After hybridization, the CD is washed four times for 1 min with 10 mM maleate buffer containing 15 mM NaCl and 0.1% Tween® 20, pH 7.5. The CD is incubated with a solution of streptavidin-colloidal gold conjugate and then with the Silver Blue solution (AAT, Namur, Belgium) as described earlier (1). The results are digitized and quantified with software that is included in the workstation (AAT).

Bio-CD Reader

A commercially available CD reader (Creative Laboratories, Singapore, Singapore) was used to read the numeric information written onto the CD. A second laser-based reader was attached on the upper part of the CD reader. The reader consists of a laser diode module that illuminates a 50- μm spot on the surface of the CD with a wavelength of 670 nm (Figure 3). The diffracted light is detected by a photodiode, and the data are digitized by an acquisition card (National Instruments, Austin, TX, USA).

The head of the detector moves following a stepping motor-driven radial displacement with a speed of 20 mm/min while the CD is turning. The overall CD surface devoted to the DNA analysis is a 15-mm-wide external band. This surface is scanned in less than 1 min, and the overall digitized data represent 6 MB of information. The data from each array are retreated to recreate the picture of each array present on the CD. Each array is stored in a separate file. Image analysis is then processed by the evaluation of the average gray level of the pixels of each spot, minus the average gray level of pixels surrounding the spot. The means of quadruplates are then calculated \pm SD.

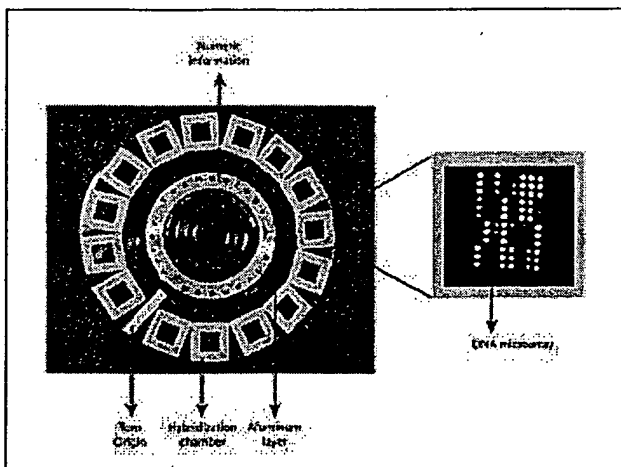


Figure 1. View of a CD used for microarray detection. The center contains numeric information covered by an aluminium layer. The outer part of the CD is covered by 15 hybridization chambers in which a microarray has been spotted.

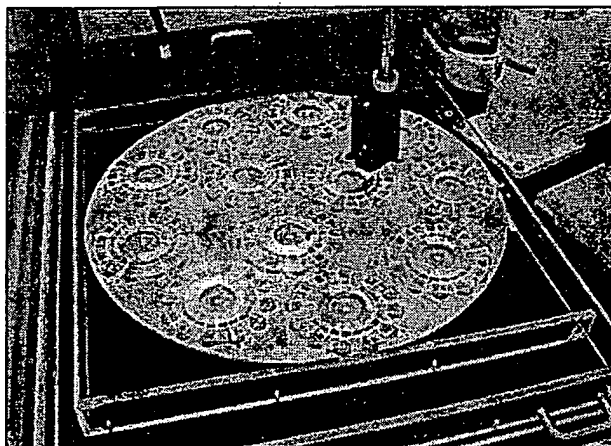


Figure 2. Axial arrayer developed for the transfer of capture probes present in solution from the multi-well plate onto the surface of the CD. The arm of the robot is a θ -based movement, and the disc is fixed on a rotating platform that has 12 CDs.

RESULTS

The optimization of the conditions for silver precipitation and reading with the double CD reader were first performed on biotinylated probes fixed on the CD. Once the detection was optimized, the CD platform was tested using the DNA hybridization assay.

A normal CD reader was used to read the numeric information on the inner part of the CD. This commercial CD reader also controlled the CD rotation speed during the analysis. The analysis of the outer part of the CD carrying the genomic results was performed by a second laser-based reader (Figure 3), which scans the outer part of the CD and measures the scattered light caused by silver precipitates that result from positive hybridization.

The signal was digitized by a PC acquisition card and stored as files, each one corresponding to one array. The data acquisition was then followed by the use of specific data analysis and data mining software.

The CD detection method was first tested for the detection of *Staphylococci* strains as already developed on glass (8). The principle of the method is to amplify a part of the *fem A* gene that is present in all *Staphylococcus* species using consensus primers and to detect them by hybridization on capture probes specific for each of the species. This array detects the five most common *Staphylococci* species. The array also contained a consensus capture probe for the genus *Staphylococcus* identification and a capture probe for the *mec A* gene. The *mec A* gene is associated with the methicillin resistance of the *Staphylococcus*. The image of each array is reconstituted, and the spot analysis was processed as followed. The program first identifies the spots and corrects for the deformation of the rectangular shape of the array given the circular reading on the CD. The average intensity of each spot inside its boundary is then calculated. The mean value of the background around

each spot is subtracted from the spot values. The means of the replicates are then calculated with two standard deviations, and values are assigned to the sequence of a specific bacteria.

A first comparative assay for the detection of the *fem A* and *mec A* sequence from methicillin-resistant *S. epidermidis* on glass slide and on CD is presented in Figure 4. The positive signals are in dark on the glass, resulting from light absorption of the illuminated glass, while they appear as bright signal on the CD because of the laser diffraction detected by the photodiode. Besides this difference, both patterns of hybridization were similar with the *S. epidermidis*, the consensus, and the *mec A* spots being positive on both arrays.

The experiment was then extended with a single CD spotted with 10 arrays. Hybridization chambers were stuck around each array. *Fem A* and *mec A* sequences from nine *Staphylococci* species were then amplified by a duplex PCR using the consensus primers and products hybridized on the arrays. A negative PCR control was also added. After silver precipitation, the arrays were analyzed in the double CD reader, which was inserted into a computer (Figure 5). These reconstituted data were processed, and the quantification of these 10 arrays obtained after image analysis is shown (Figure 6).

All the detections were specific for their respective products. The consensus *fem A* capture probe and the *mec A* were positive for all the samples, while the specific capture probes only detect their respective products, such as *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and *S. saprophyticus*. The quantitative data confirmed such conclusions because the signal-to-noise ratio was above 50 for all positive signals.

DISCUSSION

The CD is probably one of the most commonly used data storage support in our daily lives. Here we demonstrated that a CD platform could be adapted for performing DNA analysis while keeping its powerful storage of numeric information. Coupling both genomic and numeric information was achieved through the use of the same physical CD support.

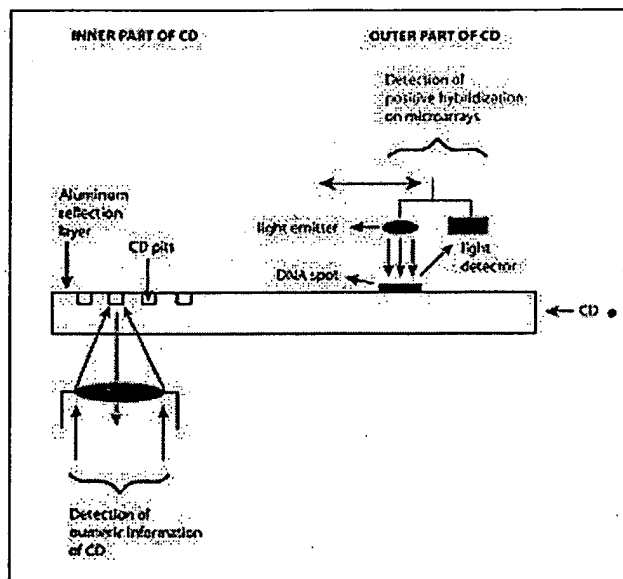


Figure 3. Schematic representation of a reading system for colorimetric CD detection composed of two laser-based devices. The first one is a classic laser CD reader reading the numeric information registered in the inner phase of the CD, and the second one is a photodiode head detecting the laser light diffraction in the presence of positive hybridization onto the microarray of the CD.

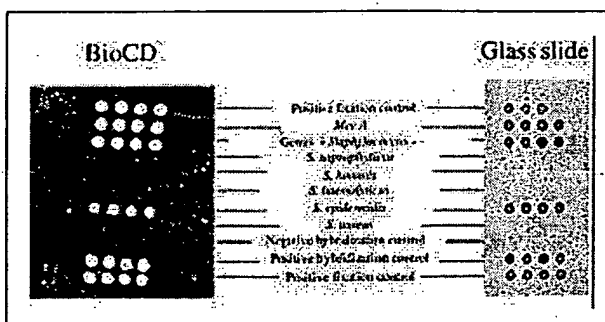


Figure 4. Result of the hybridization of a duplex PCR product made on two plasmids, one containing the *fem A* gene of *S. epidermidis*, and the other one containing the *mec A* gene. The hybridization was performed on a microarray on the surface of a CD and on a glass slide. The array is composed of 11 lines and four columns; each line is composed of four spots of the same DNA capture probes. The sequences detected by the various capture probes are given in the figure. The genus *Staphylococcus* probe recognized the bacteria from *Staphylococcus* genus, and the other five capture probes are specific to five *Staphylococcus* species. Hybridization was performed as described earlier (8). The biotinylated products were incubated with streptavidin-gold conjugate and then with the Silver Blue solution for silver precipitation and colorimetric detection (1).

The inner part of the CD contains all the registered information needed for performing the CD reading and controlling the CD reader. This information also provides the location and identification of the various arrays and the DNA capture probes present on the CD. In addition, it can include the quantification program necessary for the data management. Recordable area is yet another possible alternative for writing and storing the biological results of the microarrays on the CD.

One of the main advantages of the CD is its very large surface, which can afford many different arrays or a few very large ones. Figure 1 shows a CD that has a working surface of 74.5 cm² for biological analysis. Using spots of 0.2 mm every

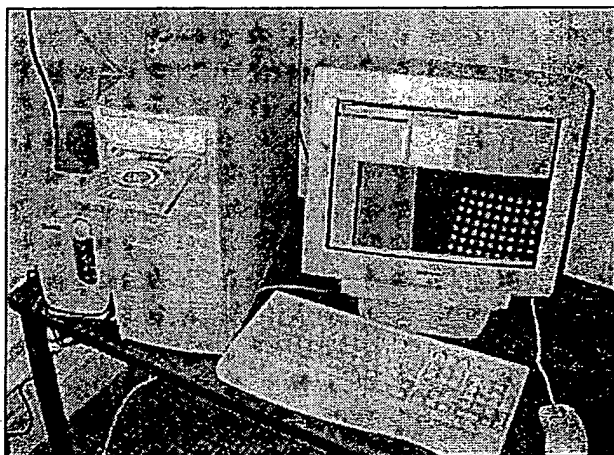


Figure 5. Photograph of the double-sided CD detector. It is composed of a normal laser CD reader to read the numeric information of the CD and a second laser head to detect the presence of positive DNA hybridization onto the microarray of the Bio-CD.

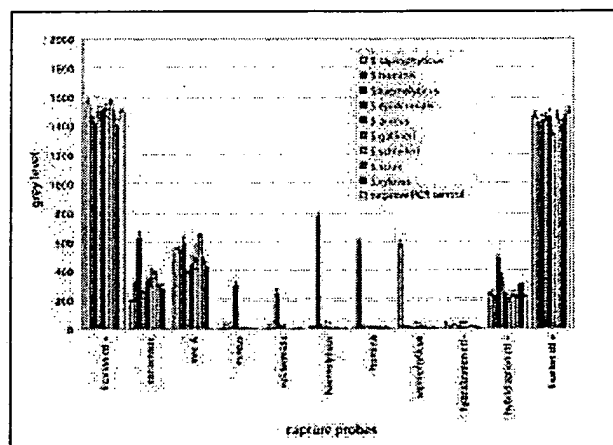


Figure 6. Results of the hybridization of nine duplex PCR products made on nine plasmids containing *fem A* from nine different *Staphylococcus* species in the presence of a second plasmid containing the *mec A* gene. The assay also contains one negative PCR control. The hybridizations were made on 10 microarrays present on the same disc platform. Detection is performed with silver precipitation technology. The nine species are *S. aureus*, *S. epidermidis*, *S. gallinarii*, *S. hominis*, *S. saprophyticus*, *S. schleiferi*, *S. sciuri*, *S. simulans*, and *S. xylosum*. Five microliters of each PCR product were first hybridized and washed, incubated with a solution of streptavidin-gold for 45 min, and then washed again and incubated with Silver Blue solution for 10 min (1). The results show the specificity of the detection even between homologous sequences and the simultaneous detection of several samples in one incubation and reading run. The values are the $\bar{x} \pm \text{sd}$ of gray level for four spots minus the gray level of the background.

0.4 mm, it would be possible to detect 45 000 sequences on one CD. Both the CD and the CD reader inserted in the computer constitute low-cost technologies.

The fast scanning of the overall CD is another advantage of the system because it can be done within 1 min and data processing is performed automatically by the software, thus making the reading step easy for the user.

In combination with electronic and glass supports, we propose the CD as an alternative platform for making and detecting arrays that are well suited for the routine and multi-sample analysis required in diagnostic DNA and research applications.

ACKNOWLEDGMENTS

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